17th International Conference on Environmental Science and Technology

Athens, Greece, 1 to 4 September 2021

The effect of HRT in the successful bioaugmentation of CSTRs working under ammonia toxicity

TZENOS C.[1], CHRISTOU M.L.[1], KALAMARAS S.D.[1] and KOTSOPOULOS T.A.[1,*]

[1]Lab of Agricultural Constructions & Equipment, Department of Hydraulics, Soil Science and Agricultural Engineering, School of Agriculture, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece

*corresponding author:
e-mail: mkotsop@agro.auth.gr

Abstract. High levels of ammonia is a common inhibitory factor in anaerobic digestion (AD) resulting in low methane production and unbalance of the process. In the present study, the adjustment of the hydraulic retention time (HRT) and the bioaugmentation process (BP) are investigated to counter the negative effects of ammonia toxicity. Two lab scale continuously stirred tank reactors (CSTR) were operating with cattle manure at a low total ammonia nitrogen (TAN) concentration (1.8 g TAN/L). The reactors were working with 20 (R1) and 30 (R2) days HRT, respectively and ammonia toxicity conditions (6.1 g TAN/L) were achieved through the direct addition of ammonium chloride. In both reactors after the increase of TAN the average daily methane production was reduced by 37.04% in R1 and 38.52% in R2. The stepwise acclimatization of the microorganisms to high concentrations of TAN (6.5 g TAN/L) for the BP was performed in batch reactors. After the BP there was a recovery of the methane production in both reactors. In R2 the recovery was immediate, however, a delay of 20 days was observed in the recovery of R1. A likely explanation for the R1's delayed response is the slow reproduction rate of the introduced acclimatized population and the low HRT.

Keywords: Anaerobic digestion, Inhibition, Ammonia, Methane, Bioaugmentation

1. Introduction

Biogas is produced by Anaerobic Digestion (AD), a biological process occurring in the absence of oxygen (O_2). Biogas is mainly comprised of methane (CH_4) and carbon dioxide (CO_2), with concentrations in the range of 50–70% and 30–50% respectively [1]. Agro-industrial and food industry waste are commonly treated with AD, thus providing many environmental benefits [2]. Methane is produced by archaea via three pathways: aceticlastic (cleaving acetate to CH_4), hydrogenotrophic (reducing CO_2 to CH_4) and methylotrophic (converting methylated compounds to CH_4) [3].

AD is highly affected by the concentration of ammonia. At low concentrations ammonia is beneficial to the AD process, but at increased levels it can be toxic to the methanogens [4]. Ammonia in an aqueous solution has two forms, free ammonia (NH_3) and ammonium (NH_4^+). The two forms are in equilibrium, the balance of which is affected by temperature and pH [5].

$$NH_3(aq.)+H_2O(l.)\leftrightarrow NH_4^+(aq.)+OH^-(aq.) \quad (1)$$

Of the two forms, free ammonia in high concentrations acts mainly as an inhibitor to the methanogenic phase of AD [6].

There are multiple methods to mitigate the inhibitory effect of ammonia in AD, such as the use of inorganic additives ($HMgPO_4$, zeolite), regulation of temperature and of the pH value, substrate dilution and carbon/nitrogen ratio increase (C/N) [7]. An innovative method of overcoming ammonia inhibition is the process of bioaugmentation, the addition of resistant - acclimated microorganisms to high levels of ammonia, directly to the bioreactor [8]. Multiple studies support the success of the BP in alleviating the effects of ammonia toxicity in CSTRs [6, 9]. However, the influence of HRT on the effectiveness of the BP has not been sufficiently investigated.

2. Materials and methods

2.1. Inoculum and substrate

The inoculum was procured from a mesophilic (37 ± 1°C) biogas plant in Central Macedonia, Greece. The substrate consisted of cattle manure from a dairy farm in the Lagada region, Thessaloniki, Greece. After sieving the cattle manure to prevent clogging, it was homogenized and stored at −20 °C. The manure was thawed for 3 days at 4°C before use. The characteristics of both the inoculum and manure are presented in Table 1.

Table 1. Manure and inoculum characteristics

	Manure	Inoculum
Total Solids (TS) (g/L)	40.3 ± 0.016	26.6 ± 0.019
Volatile Solids (VS) (g/L)	30.9 ± 0.012	20.2 ± 0.048
TAN (g/L)	44.01 ± 1.85	221.21 ± 8.64
Total VFA (mg/L)	7227.48 ± 20.48	104.24 ± 2.65

2.2. Experimental setup and operation

2.2.1. CSTR reactors

Two identical lab-scale CSTR reactors, R1 and R2 were used. The total and working volume for each reactor was 2 L and 1.5 L, respectively. Both reactors operated under mesophilic condition (37 °C) and continuous mechanical stirring (Stuart, stir UC151). To maintain a constant temperature of 37 °C (±0.1) hot water was circulated inside the internal inox spiral heat exchanger of the reactors. Two peristaltic pumps (Cole-Parmer Masterflex L/S and Cole-Parmer Masterflex Console Drive) were supplying the reactors with substrate twice per day. The setup of each reactor also included a glass influent bottle with a magnetic stirrer to homogenize the substrate, an effluent bottle, and an automatic water displacement gas volumetric meter.

2.2.2. Stepwise acclimatization

The gradual acclimatization (Stepwise Increase) of microorganisms to increasing ammonia concentrations was carried out in batch reactors with a total volume of 2 L. The batch reactors were flushed with pure nitrogen gas to create anaerobic conditions and sealed. Their temperature was maintained at a constant range of 37 ± 1 °C. Gradually the concentration of TAN was increased from 2.0 g L^{-1} to 6.5 g L^{-1} (with 0.5 g L^{-1} steps).

2.2.3. Experimental design and operation

For the duration of the experiment the two reactors operated continuously with an organic loading rate (OLR) of 1.03 gVS L^{-1} d^{-1}. Both reactors were filled with inoculum up to their working volume and were initially fed only with cattle manure with an HRT of 30 days. After steady state conditions (less than 10% variation in methane production for 10 days) were reached in both reactors their operation was continued for 30 days. The substrate for R1 was diluted with water, 2:1 volumetric ratio (cattle manure:water), in order to decrease the HRT from 30 to 20 days while maintaining a constant OLR of 1.03 gVS L^{-1} d^{-1}. The reactors operated for 30 days under steady state conditions after the HRT change in R1. For the rest of the experiment R1 operated with a 20 day HRT and R2 with a 30 day HRT. The HRT and substrate characteristics are presented in Table 2.

Table 2. Reactor HRT and substrate characteristics

	R1	**R2**
HRT (day)	20	30
Substrate (% vol.) (Manure / Water)	66.6 / 33.3	100 / 0

The TAN concentration, after the HRT change and at steady state conditions, was 1.8 g L^{-1} in both reactors. Phase 1 (P1) of the experiment begun with the direct addition of ammonium chloride (NH_4Cl, Sigma Aldrich, purity 99.998%) to both reactors and substrate, the TAN concentration was increased to 6.1 g L^{-1}. TAN concentrations higher than 5 g L^{-1} are expected to induce ammonia toxicity and inhibit the AD process [5]. After steady state conditions were reached in both reactors their operation continued under ammonia toxicity conditions for 30 days.

To implement the BP the inoculum from the batch reactors, containing the acclimatized population, was condensed via centrifuge (4500 rpm for 10 minutes at 21 °C). At the beginning of Phase 2 (P2) 180 mL from each reactor were replaced with the same volume of the condensed bioaugmentation inoculum. Both reactors operated for 30 days after the BP with a constant TAN concentration of 6.1 g L^{-1}.

There are two phases to the experiment: (a) Phase 1 (P1), operation under ammonia toxicity conditions and (b) Phase 2 (P2), operation after the BP.

2.3. Analytical methods

Total solids (TS), volatile solids (VS) and TAN were determined based on APHA's standard methods [10]. A bench digital pH meter (JENWAY 3520, Essex, UK) was used to perform daily measurements.

The CSTR reactors' biogas production was monitored daily using the automatic water displacement gas volumetric meters. To determine the biogas composition, a gas chromatograph (GC-2010plusAT, Shimadzu, Kyoto, Japan) was used equipped with the Thermal Conductivity Detector (TCD) and 2 connected columns [4]. In batch reactors daily measurements of methane concentration were taken and the methane production volume was calculated via headspace pressure measurements with the GC (TCD) [4]. Samples were obtained from all the reactors with a gas-tight syringe outfitted with a pressure lock and a needle and they were injected into the chromatographer.

Samples for Volatile Fatty Acid (VFA) measurement were obtained daily and were analysed using a gas chromatograph (GC-2010plusAT, Shimadzu, Kyoto, Japan) equipped with a Flame Ionization Detector (FID) and a single column [11].

3. Results and discussion

3.1. Operation under ammonia toxicity

The daily methane production in mL CH_4 gVS^{-1} d^{-1} of both reactors is presented in Figure 1 and the total VFA concentration in mg L^{-1} and pH values in Figure 2. During the steady state operation, prior to ammonia toxicity (P1), the average daily methane production was 236 ± 20 mL gVS^{-1} d^{-1} and 236 ± 20 mL gVS^{-1} d^{-1} for R1 and R2, while during Phase 1, was reduced to 148 ± 15 mL gVS^{-1} d^{-1} and 145 ± 15 mL gVS^{-1} d^{-1}, respectively. In R1, a period of 20 days was required in order to resume operation in steady state conditions during Phase 1, as opposed to R2 where only a 2 day period was required. The total VFA concentration increased significantly in both reactors after the direct increase in TAN concentration resulting in the inhibition of the methanogenic archaea. In R2 a steady increase in total VFA concentration is observed until the implementation of the BP, which coincides with the reduction of methane production. However, in R1 during a period of 20 days

the total VFA concentration increases, then drops to its initial value, until its sharp growth on day 30. This 20 day period coincides with the non-steady state operation of R1 evident in Figure 1. The delay observed in the increase of total VFAs concentration in R1 can be attributed to its low HRT and the substrate's low VFA concentration. The content of R1 due to low HRT is replaced with a rapid rate contributing to the removal of VFAs from the reactor keeping their concentration low thus limiting their synergistic action with the toxicity of ammonia [12].

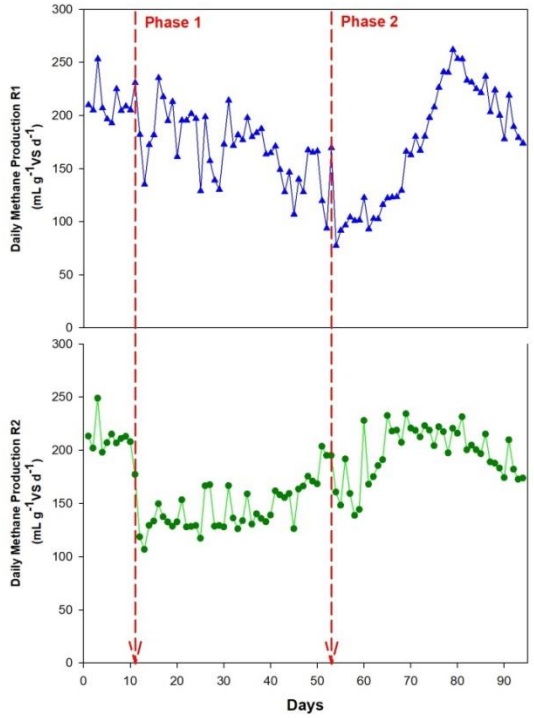

Figure 1. Daily methane production (mL CH_4 gVS^{-1} d^{-1}) for R1 and R2 reactors during Phase 1 and Phase 2

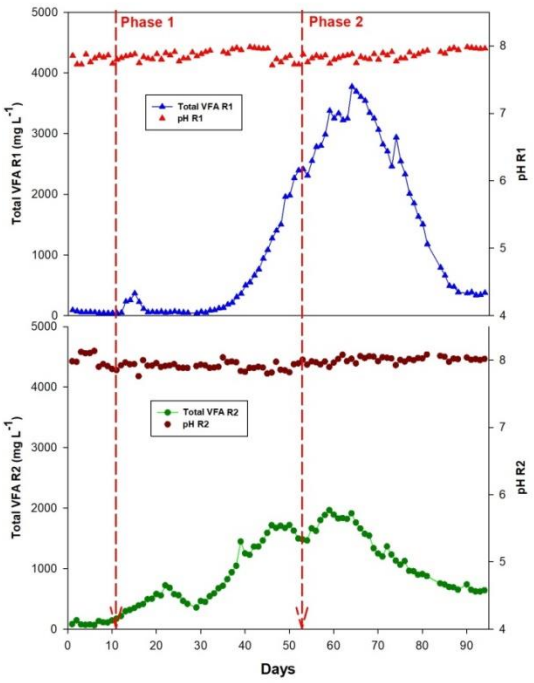

Figure 2. Total VFA concentration (mg L^{-1}) and pH values for R1 and R2 reactors during Phase 1 and Phase 2

3.2. Bioaugmentation process

After the introduction of the bioaugmentation inoculum to the reactors, each had a different response. R2 showed a direct recovery, evident from the simultaneous increase in methane production and reduction of the total VFA concentration. In contrast, the increase of methane production in R1 begun after a 10 day period. The lower HRT of R1 is probably the cause of its delayed response. The operation of R2, with 30 days HRT, provides sufficient time for the acclimatized microorganisms to establish and adapt to the reactor environment. The result of their rapid growth is the consumption of accumulated VFAs and an increase in methane production. A likely reason for the slow response of R1 to the BP is the removal of the acclimatized microorganisms due to the lower HRT (20 days), preventing their adjustment in the reactor (washout phenomenon) [13]. Under steady state conditions during Phase 2, the average daily methane production was 215 ± 20 mL gVS^{-1} d^{-1} and 214 ± 20 mL gVS^{-1} d^{-1} for R1 and R2, respectively. Therefore, the BP led to an increase in average daily methane production by 31.16% and 32.56% (8.96% and 9.40% reduction from the initial stable state) to R1 and R2, presented in Figure 3. The implementation of the BP was successful since the methane production recovered in both reactors, albeit at a slower rate in R1.

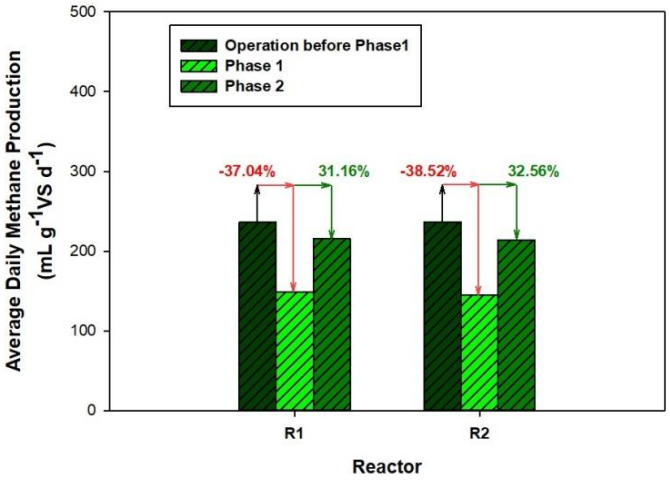

Figure 3. Average daily methane production (mL CH_4 gVS^{-1} d^{-1}) comparison for R1 and R2 reactors before Phase 1 and during Phases 1 and 2.

The pH values remained constant (8 ± 0.5) for both reactors throughout the experimental process. Maintaining a steady value in the pH scale during intense disruptions (addition of NH_4Cl and increasing the concentration of VFA), reveal the strong buffer capacity of the cattle manure. The solubility of CO_2 in the aqueous phase depends on the pH value. During pH fluctuations in the aqueous phase, the concentration of soluble CO_2 changes and maintains a stable pH value in the solution [14].

4. Conclusions

The reduction of HRT appears to influence the effectiveness of the BP adversely, probably due to the washout phenomenon of the acclimated population [15]. Therefore, a potential method to ensure that the acclimatized population remains and adapts in the bioreactor and the BP will be effective, is the increase in HRT prior to the BP in order to reduce the washout phenomenon. When the acclimatized population is firmly established in the bioreactor, the HRT can potentially be reduced to its initial value without affecting the methane production.

5. Acknowledgements

This research was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH - CREATE -INNOVATE (project code T1EDK-00406).

References

1. Angelidaki, I., L. Treu, P. Tsapekos, G. Luo, S. Campanaro, H. Wenzel, and P.G. Kougias. 2018. Biogas upgrading and utilization: Current status and perspectives. *Biotechnology Advances*, **36**(2), 452-466.
2. Achinas, S. and G.J.W. Euverink. 2016. Theoretical analysis of biogas potential prediction from agricultural waste. *Resource-Efficient Technologies*, **2**(3), 143-147.
3. Angelidaki, I., D. Karakashev, D.J. Batstone, C.M. Plugge, and A.J.M. Stams. 2011. Chapter sixteen - Biomethanation and Its Potential. in: *Methods in Enzymology*, (Eds.) A.C. Rosenzweig and S.W. Ragsdale, Vol. 494, Academic Press, pp. 327-351.
4. Kalamaras, S.D., S. Vasileiadis, P. Karas, I. Angelidaki, and T.A. Kotsopoulos. 2020. Microbial adaptation to high ammonia concentrations during anaerobic digestion of manure-based feedstock: biomethanation and 16S rRNA gene sequencing. *Journal of Chemical Technology & Biotechnology*.
5. Jiang, Y., E. McAdam, Y. Zhang, S. Heaven, C. Banks, and P. Longhurst. 2019. Ammonia inhibition and toxicity in anaerobic digestion: A critical review. *Journal of Water Process Engineering*, **32**, 100899.
6. Fotidis, I.A., L. Treu, and I. Angelidaki. 2017. Enriched ammonia-tolerant methanogenic cultures as bioaugmentation inocula in continuous biomethanation processes. *Journal of Cleaner Production*, **166**, 1305-1313.
7. Wang, H., I.A. Fotidis, and I. Angelidaki. 2016. Ammonia–LCFA synergetic co-inhibition effect in manure-based continuous biomethanation process. *Bioresource Technology*, **209**, 282-289.
8. Tian, H., E. Mancini, L. Treu, I. Angelidaki, and I.A. Fotidis. 2019. Bioaugmentation strategy for overcoming ammonia inhibition during biomethanation of a protein-rich substrate. *Chemosphere*, **231**, 415-422.
9. Schauer-Gimenez, A.E., D.H. Zitomer, J.S. Maki, and C.A. Struble. 2010. Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. *Water Research*, **44**(12), 3555-3564.
10. Rice, E.W., R.B. Baird, and A.D. Eaton. 2017. *STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 23RD EDITION*. *23rd ed*. American Public Health Association, American Water Works Association, Water Environment Federation.
11. Christou, M.L., S. Vasileiadis, S.D. Kalamaras, D.G. Karpouzas, I. Angelidaki, and T.A. Kotsopoulos. 2021. Ammonia-induced inhibition of manure-based continuous biomethanation process under different organic loading rates and associated microbial community dynamics. *Bioresource Technology*, **320**, 124323.
12. Lü, F., L. Hao, D. Guan, Y. Qi, L. Shao, and P. He. 2013. Synergetic stress of acids and ammonium on the shift in the methanogenic pathways during thermophilic anaerobic digestion of organics. *Water Research*, **47**(7), 2297-2306.
13. Deublein, D. and A. Steinhauser. 2010. Bioreactions. in: *Biogas from Waste and Renewable Resources*, pp. 101-109.
14. Kiyasudeen S, K., M.H. Ibrahim, S. Quaik, and S. Ahmed Ismail. 2016. An Introduction to Anaerobic Digestion of Organic Wastes. in: *Prospects of Organic Waste Management and the Significance of Earthworms*, (Eds.) K. Kiyasudeen S, et al., Springer International Publishing. Cham, pp. 23-44.
15. Fotidis, I.A., H. Wang, N.R. Fiedel, G. Luo, D.B. Karakashev, and I. Angelidaki. 2014. Bioaugmentation as a Solution To Increase Methane Production from an Ammonia-Rich Substrate. *Environmental Science & Technology*, **48**(13), 7669-7676.